

## Operation witch hunt: conjuring eradication of the parasitic red witchweed plant with trickery and potions

Joseph S. Vitelli<sup>1</sup>, Anna M. Williams<sup>1</sup>, Natasha Riding<sup>1</sup>, Annerose A. Chamberlain<sup>1</sup>, Peter Austin<sup>2</sup> and Dan Stampa<sup>2</sup>

<sup>1</sup>Biosecurity Queensland, Department of Agriculture and Fisheries, GPO Box 267, Brisbane, Queensland 4001, Australia

<sup>2</sup>Biosecurity Queensland, DAF, 30 Tennyson Street, Mackay, Queensland 4740, Australia (anna.williams@daf.qld.gov.au)

**Summary** An integrated field trial was established near Mackay, Queensland, to test treatment efficacy for eradicating the parasitic weed, red witchweed (RWW) (*Striga asiatica* (L.) Kuntze). Treatments tackling the only known infestation of RWW in Australia include pre and post-emergent herbicides, catch crop, trap crop plants and fumigants. One thousand canisters containing 5800 seed sachets were buried to monitor seed bank decline over time. Each canister contained seed sachets buried at five depths. At six months post-treatment application, 100 canisters were exhumed and a further 100 will be exhumed annually, to evaluate RWW seed viability following annual treatment applications. This trial will determine the treatment options that are most effective at accelerating soil seed bank decline and preventing above-ground emergence of RWW. These results will govern the management strategies utilised within the RWW eradication program.

The six-month retrieval data showed encouraging trends. As seed burial depth increased from 0 cm to 50 cm deep, seed viability increased from 61 to 69%. Treatments utilising herbicides did not significantly differ from the control (maintaining bare ground) treatment (81% and 77% seed viability). The catch crop, sorghum, had the biggest reduction in seed viability (17%), followed by the fumigants, dazomat (38%) and ethylene (39%). Ethylene gas has been used with considerable success in the USA for accelerating soil seed bank decline. Our preliminary data indicate, through the use of a host (false or true) plant and soil fumigant, exhaustion of the soil seed bank is achievable within three to five years.

**Keywords** *Striga asiatica*, red witchweed, parasitic weeds, eradication, field trial, seed bank.

### INTRODUCTION

*Striga asiatica* (L.) Kuntze, commonly known as red witchweed (RWW), is classified in Queensland as Prohibited Matter in Schedule 1 of the *Biosecurity Act 2014* (the Act). RWW is currently found on six properties at Habana (21°2'60"S, 149°5'60"E) west of Mackay. First recorded growing attached to sugarcane

in July 2013, an Eradication Response Plan (ERP) was submitted to the Consultative Committee for Exotic Plant Incursions (CCEPI) on 27 August 2013. The Consultative Committee for Red Witchweed agreed to a 10 year ERP, commencing on 1 July 2015, to be jointly funded by State and Federal Governments and affected industry bodies.

RWW is a small, annual plant that grows attached to the roots of a host plant (Musselman 1980). This obligate parasite (cannot develop independently from a suitable host) draws its nutrients and water from the roots of its host, generally causing the host to wilt, turn yellow and often become stunted. Hosts include a wide range of tropical and sub-tropical grass species, including several agriculturally important crops, including sugar cane (*Saccharum officinarum* L.), sorghum (*Sorghum bicolor* (L.) Moench), corn/maize (*Zea mays* L.), pearl millet (*Pennisetum americanum* (L.) Leeke) and rice (*Oryza sativa* L.) (Parker 2012).

RWW reproduces from seed and a single plant can produce between 90,000 to 450,000 seeds (Bebawi *et al.* 1984b). Bebawi *et al.* (1984a) reported that seeds could remain viable for up to 14 years when buried at a depth of 152 cm. Following seed maturation, seeds require a period of 'after-ripening' of up to two years. After this, seeds require certain soil moisture and temperature conditions for pre-conditioning ( $\geq 18^{\circ}\text{C}$ , for 12 to 21 days) and will only then germinate in the presence of a germination stimulant, usually exuded from a nearby host plant root (Worsham 1987). In the absence of appropriate germination conditions, seeds remain dormant but viable in the soil (Worsham 1987).

Research into effective control strategies for RWW has been undertaken by the USDA (United States Department of Agriculture) and state agricultural organisations in the USA, as well as in Africa and Madagascar with costs exceeding \$250 million in the USA alone (Sand 1987). Lessons from the Witchweed Eradication Program in the Carolinas (USA), running since 1957 (Eplee 1992), has provided the blueprint for developing eradication strategies in Queensland.

Eradication efforts in Queensland aim to incorporate techniques that accelerate RWW seed bank decline and prevent the production of new RWW seed. Ethylene-based soil fumigants are reported to trigger seed germination of RWW significantly reducing the time required to exhaust the soil seed bank to within three to five years (Bebawi *et al.* 1984). Catch crops are 'true host' species with roots that exude a stimulant to trigger germination of RWW seeds, supporting the subsequent growth of RWW through to flowering and seed-set. Trap crop plants are 'false host' species with roots that exude a stimulant to trigger germination but do not support attachment and subsequent growth of RWW. Catch crops are destroyed prior to emergence of RWW to prevent soil seed bank recruitment of the crop. This paper reports the findings of the six months exhumation on RWW seed viability following the application of pre- and post-emergent herbicides, catch crops, trap crops and fumigants to sugarcane.

#### MATERIALS AND METHODS

**Experimental site and plant material** An integrated control study was established in 2014 on one of the infested RWW properties in Habana (21°3'48"S, 149°4'21"E), Mackay, Queensland. Sugarcane is the predominant commercially viable crop grown locally. The trial will investigate the efficacy of agronomic practices for depleting the RWW seed bank and preventing production of new RWW seed, over a ten year period. The site had been planted to sugar cane and was on its eighth ratoon when RWW was discovered in July 2013. The 1.4 ha site is on an undulating rise with mostly gentle slopes of around 8 to 10% incline, with an abundance of angular stones through the profile. The soil has a coarse textured, non-coherent surface, overlying a narrow band of less permeable clay subsoil and coarser textured decomposing granodiorite.

The wet season in Habana is from December to May, with 81% of the annual rainfall falling during this period (Bureau of Meteorology 2016). The mean ( $\pm$  SEM) annual rainfall during the study period (2014 to 2015) was 1155  $\pm$  243 mm. The number of rainy days per year (where a rainy day is  $>0.2$  mm) averaged 128  $\pm$  8.

Prior to treatment application, the standing sugar cane was bumpered, slashed and ripped in January 2014, with reshooting plants sprayed with glyphosate at 1800 g a.i. ha<sup>-1</sup>. The destroyed cane was raked into mounds, left to dry and burnt in July 2014. The site was maintained fallow through regular discing, ploughing and periodic spraying with glyphosate and 2,4-D (1125 g a.i. ha<sup>-1</sup>) until planting. On the 6 August 2014, 25 of the 80 plots were furrowed, fertilised with Granam at 170 kg ha<sup>-1</sup> and planted with stalks of sugar cane

cultivar Q208. Row spacings were 1.5 m apart and ~100 m in length. The planter seed cane was sourced from Mackay Area Productivity Services.

**Experimental design and treatments** The field study to determine RWW soil seed bank run down was laid out in a 16  $\times$  5 factorial replicated five times using a randomised complete block design. Factor A was the 16 treatments (two pre- and two post-emergent herbicides, four false and two true hosts, one fertiliser and three fumigants) assigned to the mainplots, and factor B was seed burial depth (0, 10, 20, 30 and 50 cm). A perforated canister housed five seed sachets (one for each depth), with each sachet containing 100 RWW seeds. A total of 1000 canisters containing 5800 seed sachets have been buried in the field with 100 canisters to be exhumed at six months and annually thereafter to test for RWW seed viability following treatment applications. The 16 treatments were:

#### Bare ground:

Ground maintained bare through applications of 2,4-D (1125 g a.i. ha<sup>-1</sup>), paraquat (400 g a.i. ha<sup>-1</sup>) or glyphosate (1800 g a.i. ha<sup>-1</sup>) as needed;

#### Control:

Only host plant sugarcane;

#### Post-emergent herbicides applied to sugarcane:

- dicamba (300 g a.i. ha<sup>-1</sup>) – applied to sugarcane foliage every 4 to 8 weeks;
- fluroxypyr (300 g a.i. ha<sup>-1</sup>) – applied to sugarcane foliage every 4 to 8 weeks;

#### Pre-emergent herbicides applied to sugarcane:

- pendimethalin (1500 g a.i. ha<sup>-1</sup>) – applied annually in February and mechanically incorporated into soil within 5 days;
- trifluralin (1400 g a.i. ha<sup>-1</sup>) – applied annually in February to a 7.5 to 15 cm deep furrow;

#### False host plants: (hand broadcast)

- Soybean (*Glycine max*) – 50 t ha<sup>-1</sup>;
- Cowpea (*Vigna unguiculata* L.) – 90 t ha<sup>-1</sup>;
- Cotton (*Gossypium hirsutum* L.) – 15 t ha<sup>-1</sup>;
- Lablab (*Lablab purpureus* L.) – 20 t ha<sup>-1</sup>;

#### True host plants: (hand broadcast)

- Sorghum – 25 t ha<sup>-1</sup>;
- Corn – 30 t ha<sup>-1</sup>;
- Corn + urea – corn 30 t ha<sup>-1</sup> plus urea at 450 kg N ha<sup>-1</sup>;

#### Fumigants:

- Dazomat – applied annually as a granular fumigant at 330 kg a.i. ha<sup>-1</sup> to bare ground in April and immediately irrigated with 25 L water m<sup>-2</sup> to activate and form a surface crust;
- Ethephon – applied annually in April as a liquid fumigant at 70 kg a.i. ha<sup>-1</sup> to bare ground;

- Ethylene gas – Ethylene gas injected into the soil via a handheld probe to a depth of 15 to 30 cm at a rate of 2.0 kg ha<sup>-1</sup> annually in February.

**Seed sachets and canisters** All seeds used in this experiment were collected from RWW plants grown within a QC2 quarantine facility at the Ecosciences Precinct, Brisbane. After collection, approximately 500,000 seeds were removed, pooled, sieved and stored at low humidity at 35°C for 12 months as part of the seed maturation process (Worsham 1987). Only seeds >150 µm were used in this experiment. Matured seeds to be buried at 10, 20, 30 and 50cm depth were placed inside 40 mm × 65 mm 62 µm precision woven polyamide (nylon) mesh tubes (SAATIFIL PA 62/40 PW WH) with the ends sealed using self-adhesive nylon tape (PSP Spinnaker Repair Tape). Surface seeds were placed inside 40 mm × 65 mm 63 µm stainless steel woven mesh sachets and the ends sealed with a hard setting metallic filler (Selleys Metallic Fix Cement Filler). Approximately 100 seeds were removed from the seed-stock, photographed (for counting) then transferred to each sachet containing 4 g of 300 µm sieved river sand to optimise seed soil contact.

The soil canisters were made from 100 mm PVC pipe cut into 550 mm lengths and perforated using a drill press with a 10 mm drill bit. The open space for each canister ranged from 20 to 25%. The sachets were placed at depth within the canisters and filled with 10 mm sieved soil from the study site. Canister burial concluded in December 2014 with treatments commencing in January 2015.

**Laboratory procedure** Retrieved sachets were stored at low humidity at 35°C until processed. At processing, each sachet was washed to remove exterior dirt. Sachet contents were flushed into a 90 mm sieve, rinsed with water, and transferred to a 100 mL measuring cylinder to which 100 mL of potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) solution of specific gravity 1.4 was added (Eplee and Norris 1987). The cylinder was placed on a stirring plate for 30 to 45 minutes at 175 rpm to agitate the sample and encourage seeds to float to the surface. When removed from the stirring plate floating seeds were pipetted into a clean 90 mm sieve. Any seeds and debris accumulated on the edge of the cylinder were collected with a cotton bud and washed with water onto a filter paper. The remaining K<sub>2</sub>CO<sub>3</sub> was poured through the sieve without disturbing the sand. Seeds collected from the sieve were also added to the filter paper. Sand from the cylinder was further flushed onto the sieve and checked under a microscope for any seeds and seed coats. All collected seeds and seed coats were counted and intact seeds tested for

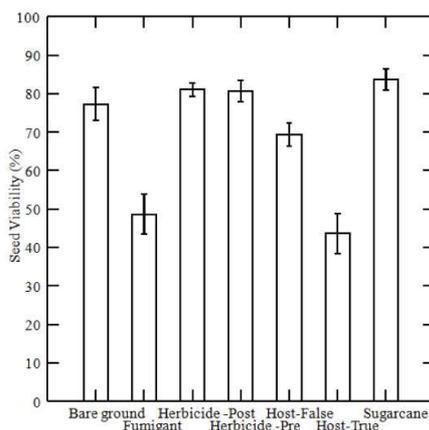
viability. Viability testing was performed using 1% 2,3,5-triphenyltetrazolium chloride (TTC) (Moore 1976). Seeds were immersed in 80 µl TTC and stored in the dark at 35°C for seven to ten days. Embryos turning red to pink in colour were considered viable.

The total number of viable seeds included those that germinated following treatment plus any ungerminated seeds identified as viable after TTC testing. An analysis of variance was performed on arcsine-transformed seed viability data and later back-transformed. Standard error of the mean (s.e.m) was used to identify differences between treatments.

## RESULTS

A significant treatment effect was observed on seed viability at six months post treatment application (P < 0.0001, Figure 1). The most effective treatments at reducing seed viability irrespective of seed burial depth were true hosts (44 ± 3% (± s.e.m) of remaining seeds viable) and fumigants (49 ± 3% (± s.e.m)), followed by false hosts (69 ± 2% (± s.e.m)). No significant difference was observed across the remaining treatments with seed viability remaining high (>77%). Within the broader category of true host sorghum was the most effective treatment with only 17 ± 3% (± s.e.m) of the buried RWW seed remaining viable after six months. The fumigants, dazomat and ethylene were equally effective at accelerating seed run down, with 38 ± 6% (± s.e.m) and 37 ± 4% (± s.e.m) of the buried seed remaining viable. Viability of laboratory stored seed over the same period was 92%.

As seed burial depth increased from 0 to 50 cm deep, irrespective of treatment, seed viability increased from 61 to 69%. However this trend was not significant at the six month period.



**Figure 1.** Effect of treatment on seed viability averaged over seed burial depth. Each column is the mean of five replicates (standard error bars shown).

## DISCUSSION

Despite the trial being in its infancy (six months), several treatments are having a significant influence at accelerating the RWW seed bank decline. The ability to optimally integrate herbicides, true and false hosts and fumigants will be critical to the successful depletion of RWW seed at infested sites.

The true host crop sorghum reduced RWW seed viability to 17% markedly lower than the bare ground control (77%). Sorghum unfortunately presents a risk to the sugar cane production system, as an additional weed to manage if allowed to seed, with a reported seed longevity of up to 13 years (Burnside *et al.* 1977). Further, if sorghum was left unchecked, RWW would continue to emerge and set seed exacerbating the RWW problem. In West Africa, Murdoch and Kunjo (2003) reported that in systems where *Striga* was allowed to co-exist without chemical control, the soil seed bank of *Striga hermonthica* increased 270% in two wet seasons.

Dazomat is an effective dry formulation fumigant, with treatment application resulting in 3% of surface buried RWW seed remaining viable after six months. Wide scale use of dazomat offers challenges, both in cost (\$3777 ha<sup>-1</sup>) and the need for the product to be soil incorporated with water (25 mm rainfall or irrigation) within a few days after application, when soil temperatures are less than 10°C. The soil injection of ethylene gas saturates the soil profile, initiating germination of all seed that are in a pre-conditioned state, and is very effective at depth with only 35% of RWW seed viable at 50 cm burial after six months. However, the injection of ethylene by hand takes 85 h ha<sup>-1</sup> to apply. Adoption of ethylene as an eradication tool necessitates the need for fabricating a mechanised system.

In the interim, two Emergency Use Permits PER14357 (<http://permits.apvma.gov.au/PER14357.PDF>) and PER14361 (<http://permits.apvma.gov.au/PER14361.PDF>) are currently in place to assist eradication of RWW in Queensland. To date, eradication efforts outside the trial site are incorporating herbicides, the false host soybean and ethylene injection. Findings from our research will allow site specific control strategies to be developed, hastening RWW soil seed bank extirpation.

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